p7.5tk

7.5 k promoter

Not I Apa I

GGCCAAAAATTGAAAAACTAGATCTATTTATTGCACGCGGCCGCCATG GGC CCG GCC GCC AAC GGC GGA

Met Gly Pro Ala Ala Asn Gly Gly

tk coding sequence

pE/Ltk

synthetic E/L promoter

Not I Apa I

GGCCAAAAATTGAAATTTTATTTTTTTTTTTGGAATATAAAGCGGCCGCCATG GGC CCG GCC GCC AAC GGC GGA

Met Gly Pro Ala Ala Asn Gly Gly

tk coding sequence

FIGURE 1

A

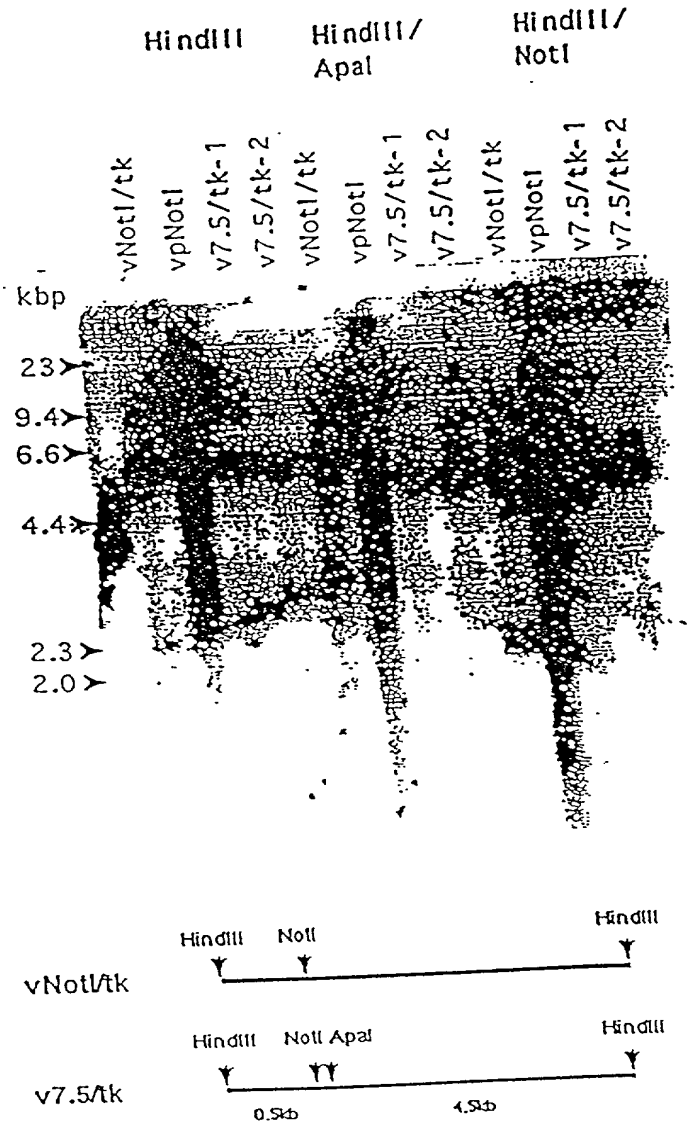

HindIII
HindIII/ ApaI
HindIII/ NotI
vNotI/tk
vpNotI
v7.5/tk-1
v7.5/tk-2
vNotI/tk
vpNotI
v7.5/tk-1
v7.5/tk-2
vNotI/tk
vpNotI
v7.5/tk-1
v7.5/tk-2
kbp
23
9.4
6.6
4.4
2.3
2.0
HindIII
NotI
HindIII
vNotI/tk
HindIII
NotI ApaI
HindIII
v7.5/tk
0.5kb
4.5kb

FIGURE 2 A

B

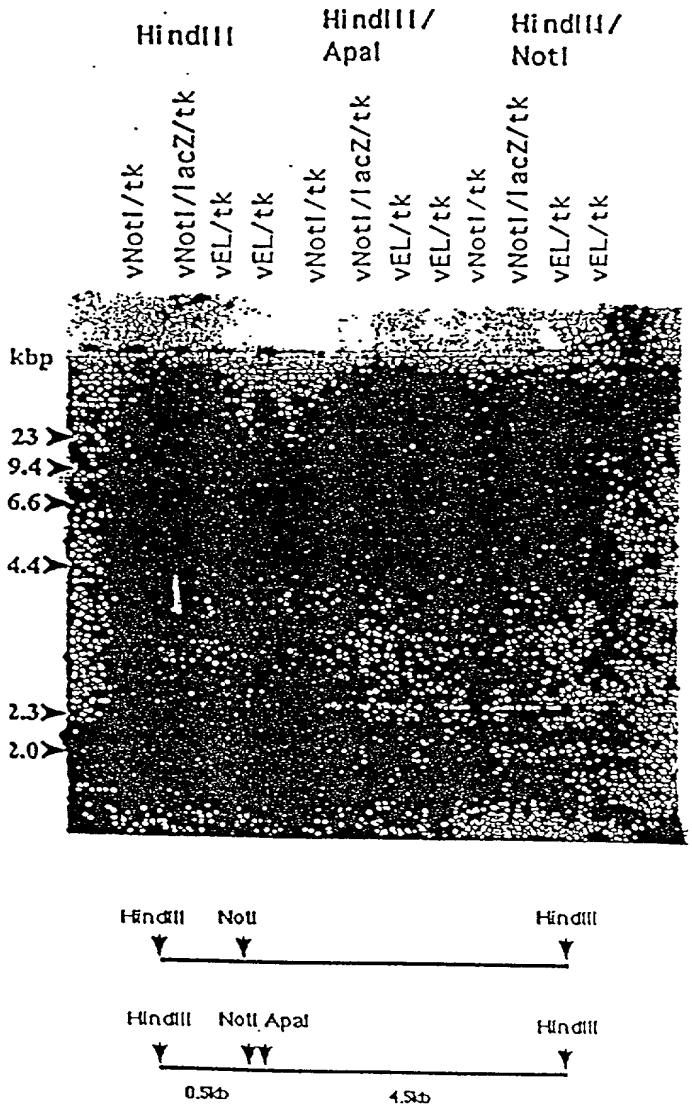

HindIII
HindIII/ ApaI
HindIII/ NotI
vNotI/tk
vNotI/lacZ/tk
vEL/tk
vEL/tk
vNotI/tk
vNotI/lacZ/tk
vEL/tk
vEL/tk
vNotI/tk
vNotI/lacZ/tk
vEL/tk
vEL/tk
kbp
23
9.4
6.6
4.4
2.3
2.0
HindIII
NotI
HindIII
HindIII
NotI ApaI
HindIII
0.5kb
4.5kb

FIGURE 2B

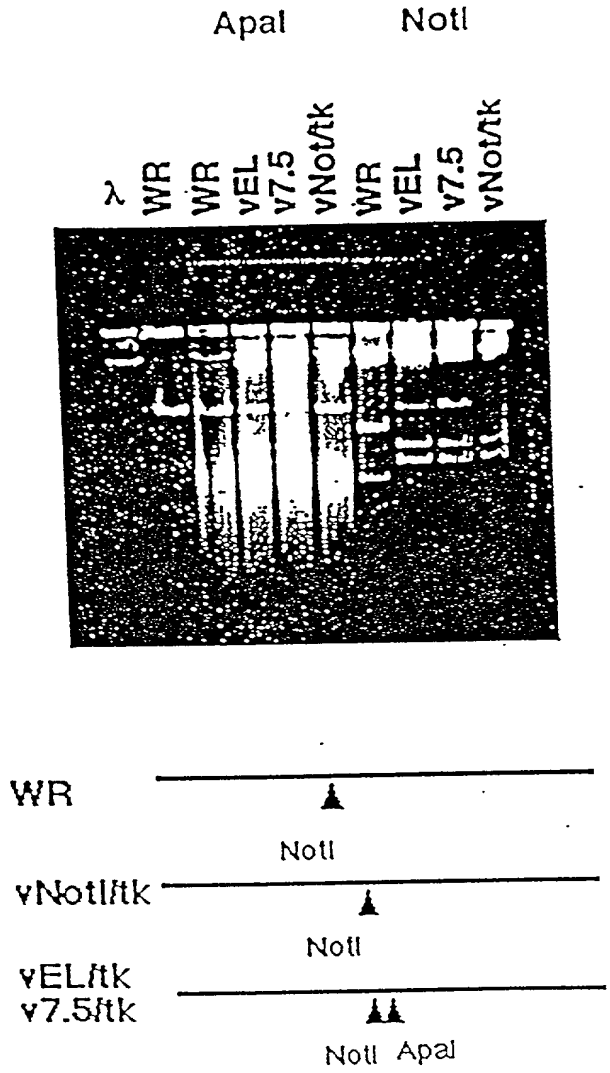

ApaI
NotI
λ
WR
WR
vEL
v7.5
vNot/tk
WR
vEL
v7.5
vNot/tk
WR
NotI
vNotI/tk
NotI
vEL/tk
v7.5/tk
NotI ApaI

FIGURE 3

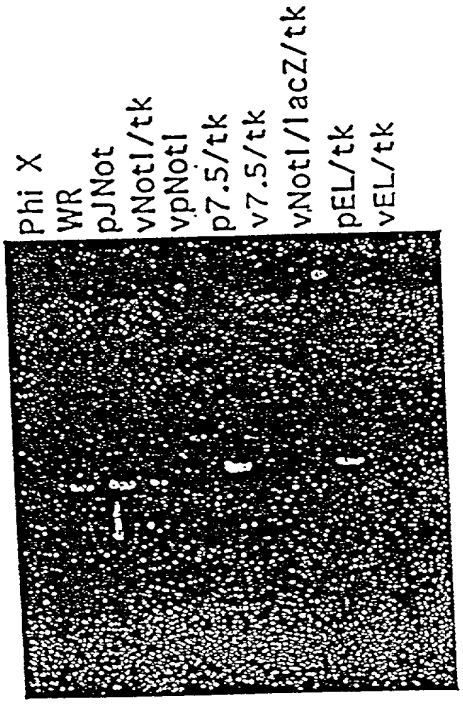

Phi X
WR
pJNot
vNotI/tk
vpNotI
p7.5/tk
v7.5/tk
vNotI/lacZ/tk
pEL/tk
vEL/tk

FIGURE 4

B-Glucoronidase Analysis of Recombinant Viruses

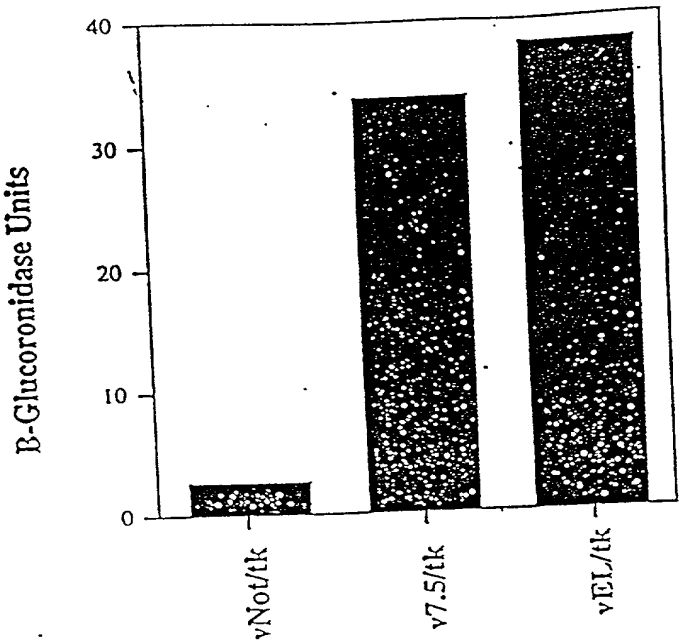


FIGURE 5

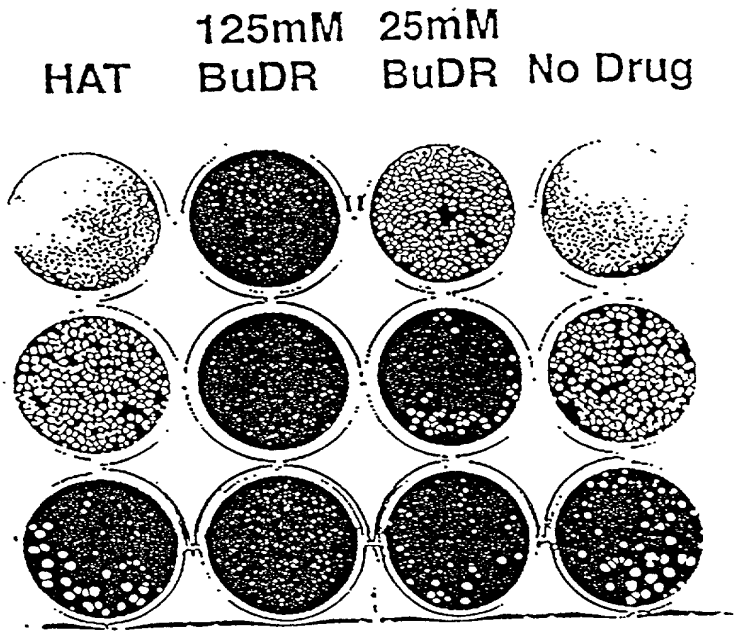


FIGURE 6

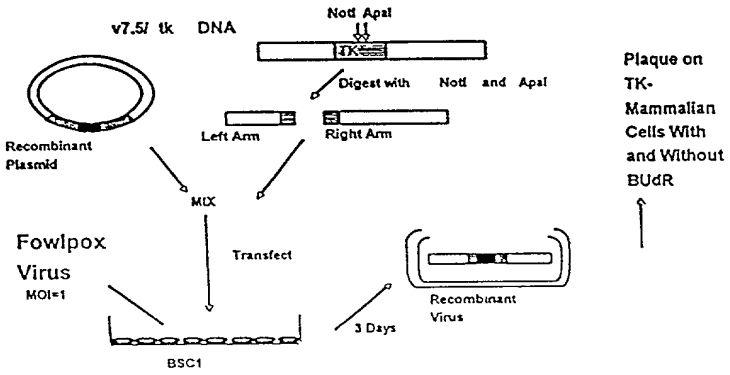

v7.5/ tk DNA
NotI ApaI
TK
Digest with NotI and ApaI
Left Arm
Right Arm
Recombinant Plasmid
MIX
Transfect
Fowlpox Virus
MOI=1
BSC1
3 Days
Recombinant Virus
Plaque on TK- Mammalian Cells With and Without BUdR

FIGURE 7

1. p7.5tk

7.5K PROMOTER NOTI APAI

5'- GGCCAAAAATTGAAAAACTAGATCTATTTATTGCACGCGGCCGCCATGGGCCCGGCC -3'

2. p7.5/ATG0/tk

7.5K PROMOTER NOTI BAMHI SMAI PSTI

5'- GGCCAAAAATTGAAAAACTAGATCTATTTATTGCACGCGGCCGCCGTGGATCCCCCGGGCTGCAGGAA

TRANSLATION TRANSCRIPTION

STOP CODONS STOP SIGNAL

SALI

TTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCCTAACTAACTAATTTTGTTTTTGT

APAI

GGGCCCGGCC -3'

FIGURE 8A

3. p7.5/ATG1/tk

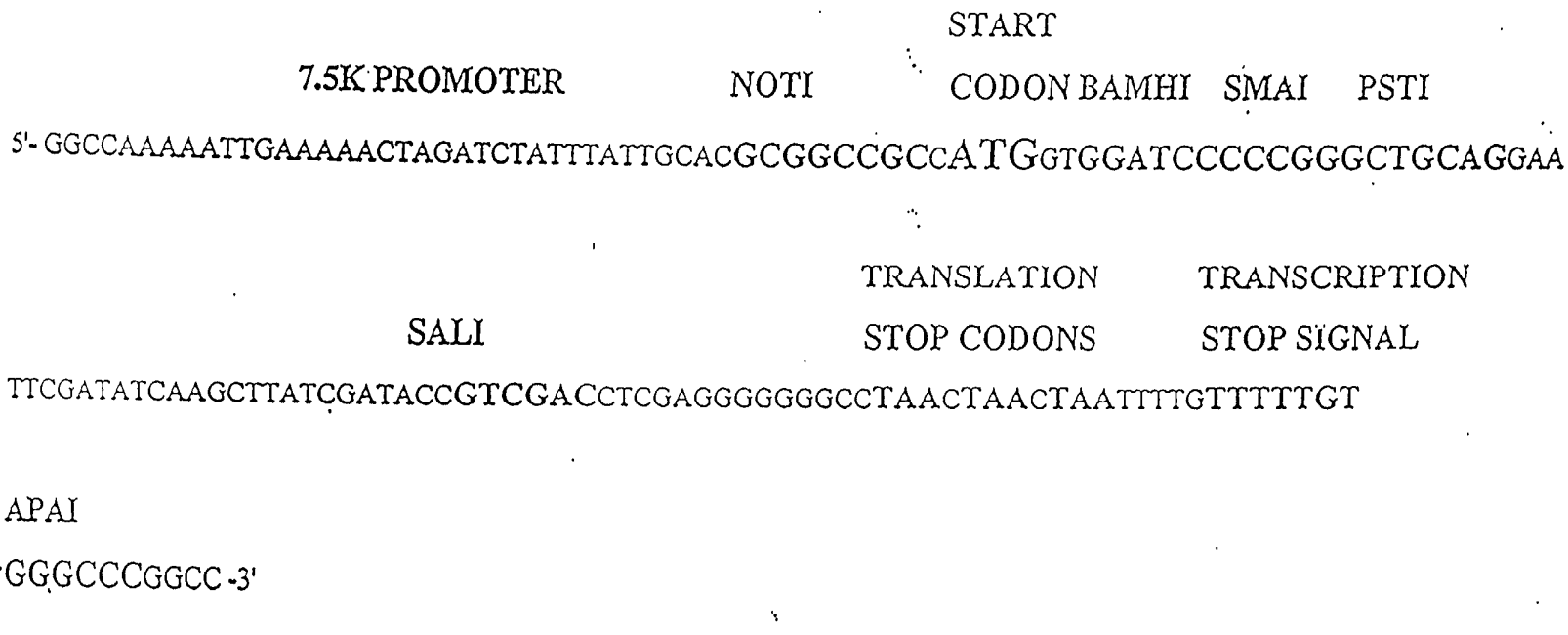


FIGURE 8B

4. p7.5/ATG2/tk

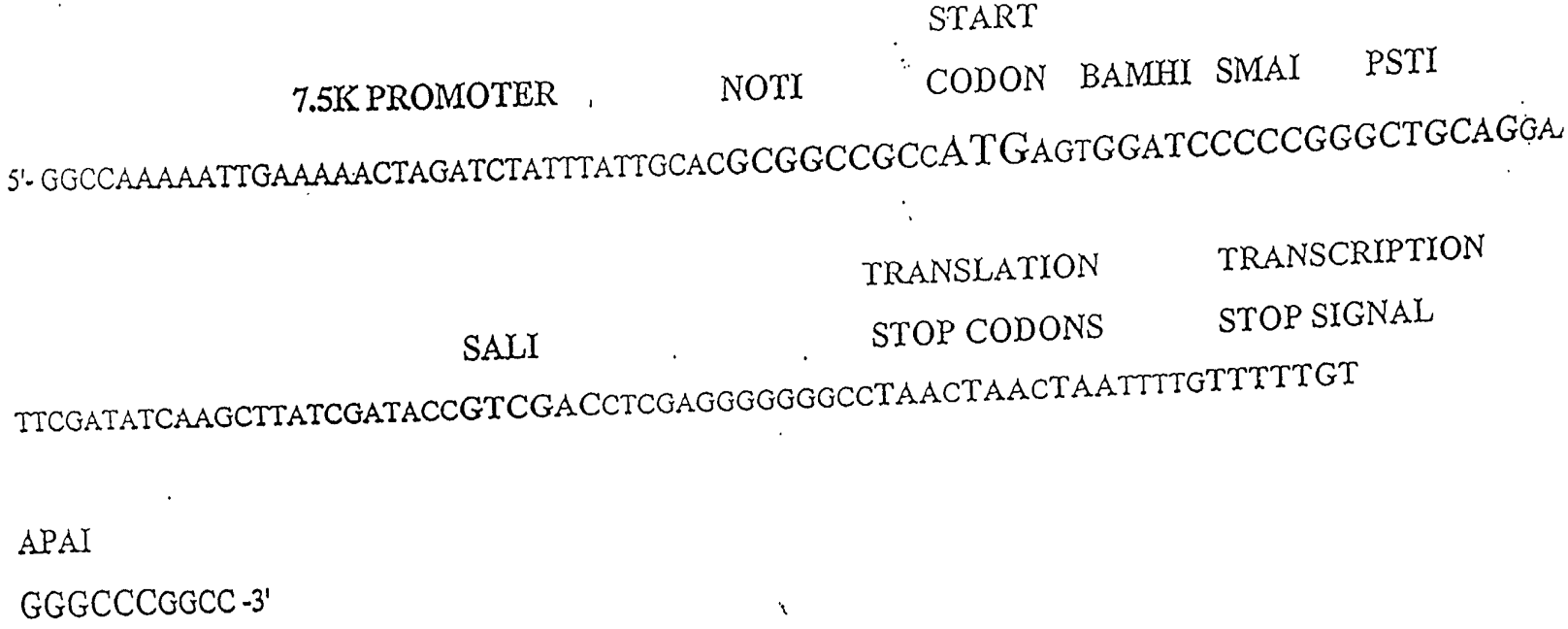


FIGURE 8C

5. p7.5/ATG3/tk

START

7.5K PROMOTER NOTI CODON BAMHI SMAI PSTI

5'- GGCCAAAAATTGAAAAACTAGATCTATTTATTGCACGCGGCCGCCATGACGTGGATCCCCCGGGCTGCAGGAA

TRANSLATION TRANSCRIPTION

STOP CODONS STOP SIGNAL

SALI

TTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCCTAACTAACTAATTTTGTTTTTGT

APAI

GGGCCCGGCC -3'

FIGURE 8D

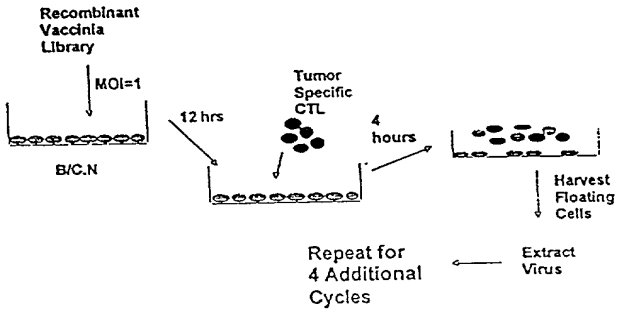

Recombinant
Vaccinia
Library
MOI=1
B/C.N
12 hrs
Tumor
Specific
CTL
4
hours
Harvest
Floating
Cells
Repeat for
4 Additional
Cycles
Extract
Virus

FIGURE 9

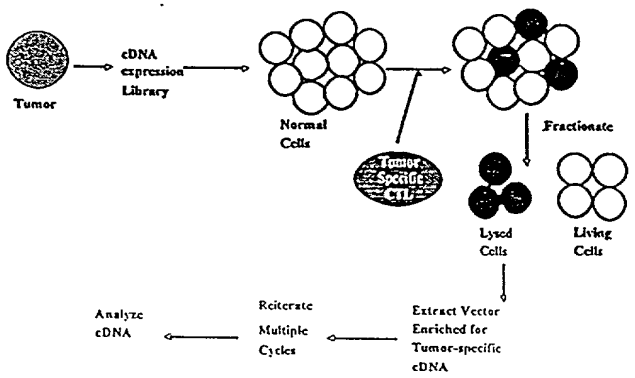

Tumor
cDNA
expression
Library
Normal
Cells
Tumor
Specific
CTL
Fractionate
Lysed
Cells
Living
Cells
Extract Vector
Enriched for
Tumor-specific
cDNA
Reiterate
Multiple
Cycles
Analyze
cDNA

FIGURE 10

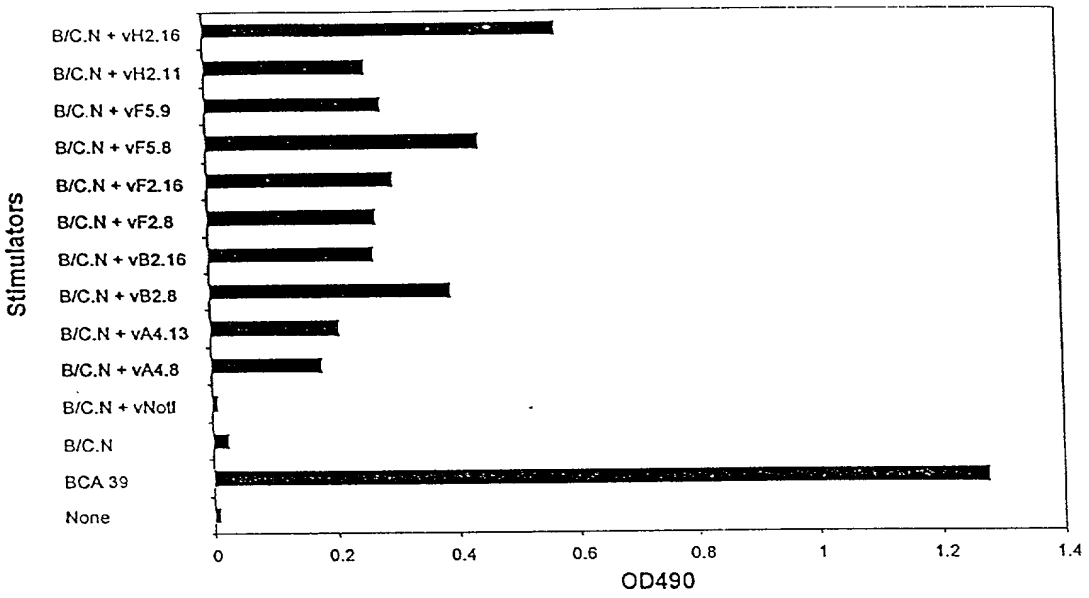

Stimulators
B/C.N + vH2.16
B/C.N + vH2.11
B/C.N + vF5.9
B/C.N + vF5.8
B/C.N + vF2.16
B/C.N + vF2.8
B/C.N + vB2.16
B/C.N + vB2.8
B/C.N + vA4.13
B/C.N + vA4.8
B/C.N + vNotI
B/C.N
BCA 39
None
0
0.2
0.4
0.6
0.8
1
1.2
1.4
OD490

FIGURE 11A

Target	Percent Specific Lysis Effector : Target 10:1	2:1
BCA 34	68.4	54.8
BCA 39	36.6	23.4
B/C.N	0.2	0.3
B/C.N + vF5.8	47.5	34.6
B/C.N + vH2.16	67.8	56.2
B/C.N + vaccinia vector	0	0.2

FIGURE 11B

A. L3

Amino Acid Position	45	46	47	48	49	50	51	52	53	54	55	56
Sequence	A	F	L	G	Y	K	A	G	M	T	H	I
Nucleotide	GCC	TTT	CTG	GGT	TAC	AAG	GCT	GGC	ATG	ACc	CAC	ATC

B. H2.16

Amino Acid Position	45	46	47	48	49	50	51	52	53	54	55	56
Sequence	A	F	L	G	Y	K	A	G	M	I	H	I
Nucleotide	---	---	---	---	---	---	---	---	---	-T-	---	---

FIGURE 12

Target	Percent Specific Lysis Effector: Target 10:1	2:1
BCA 34	62.4	32.1
BCA 39	49.7	23.6
B/C.N	3.3	0.2
B/C.N + L3 peptide 48-56(I54)	46.0	16.1
B/C.N + L3 peptide 48-56(T54)	2.0	0
B/C.N + L3 peptide 45-54(I54)	0	0

FIGURE 13A

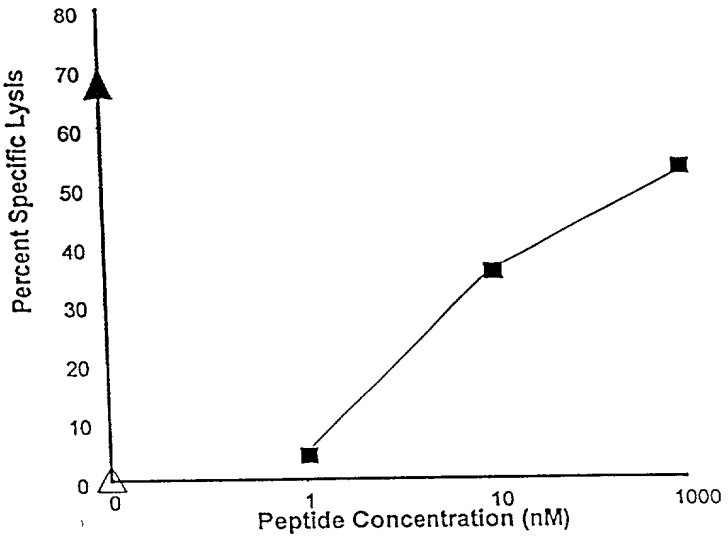
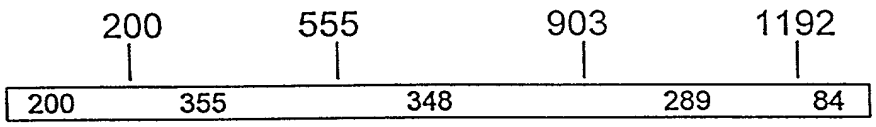

80
70
60
50
40
30
20
10
0
Percent Specific Lysis
0
1
10
1000
Peptide Concentration (nM)

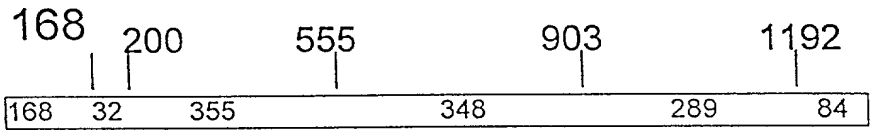
FIGURE 13B

Published L3 (1276 bp)



168-171 = GACC

H2.16 (1276 bp)



168-171 = GATC

FIGURE 14A

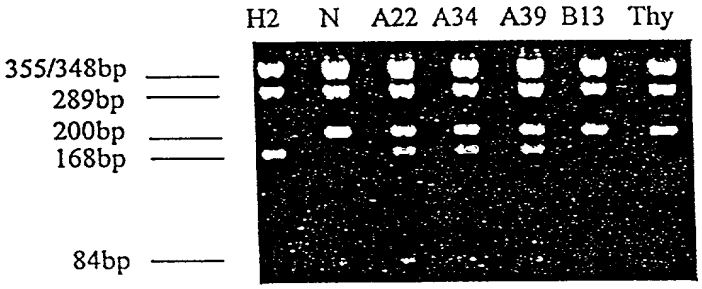


FIGURE 14B

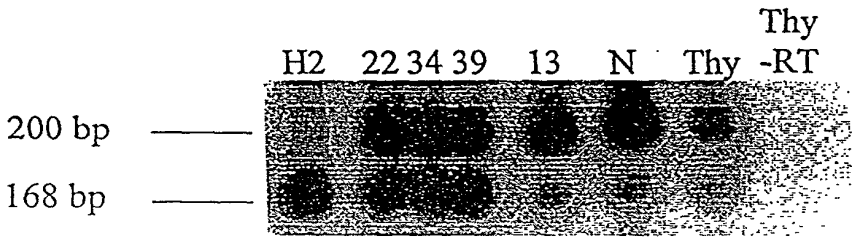


FIGURE 14C

	Percent Specific Lysis Immunogen			
	vH2.16		v7.5/tk	
Target	40:1	10:1	40:1	10:1
BCA 34	33.6	12.9	5.7	4.0
BCA 39	22.1	9.0	5.3	3.1
B/C.N + L3 48-56 (I54)	48.2	20.2	3.9	1.5
B/C.N + L3 48-56 (T54)	6.4	1.4	1.8	2.9
B/C.N	7.1	5.7	6.1	2.8
YAC	1.2	2.5	0	1.8

FIGURE 15 A

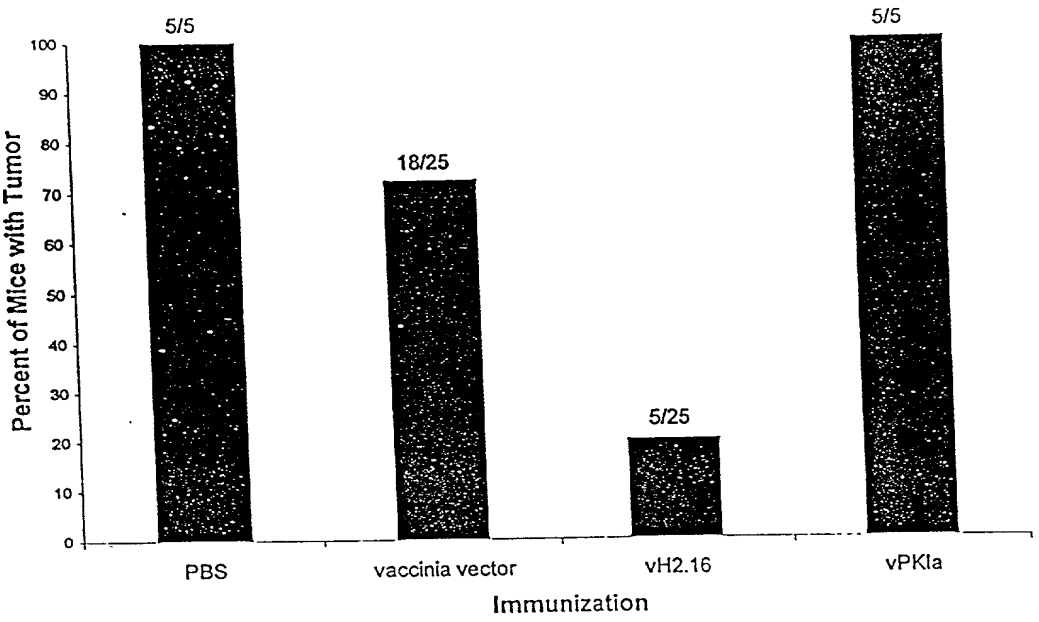

5/5
18/25
5/25
5/5
100
90
80
70
60
50
40
30
20
10
0
Percent of Mice with Tumor
PBS
vaccinia vector
vH2.16
vPKIa
Immunization

FIGURE 15B

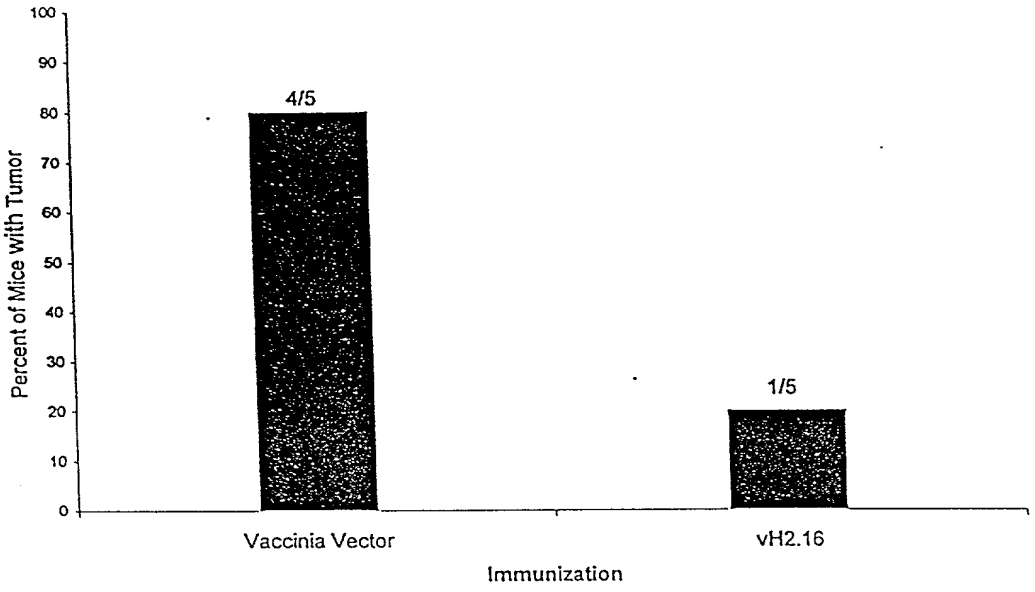

Percent of Mice with Tumor
100
90
80
70
60
50
40
30
20
10
0
4/5
1/5
Vaccinia Vector
vH2.16
Immunization

FIGURE 15C

A.

Influenza Specific Cytolytic Activity of CD4+ CD45RA+ Human T Cells Stimulated in the Presence of IL-12 and IL-18.

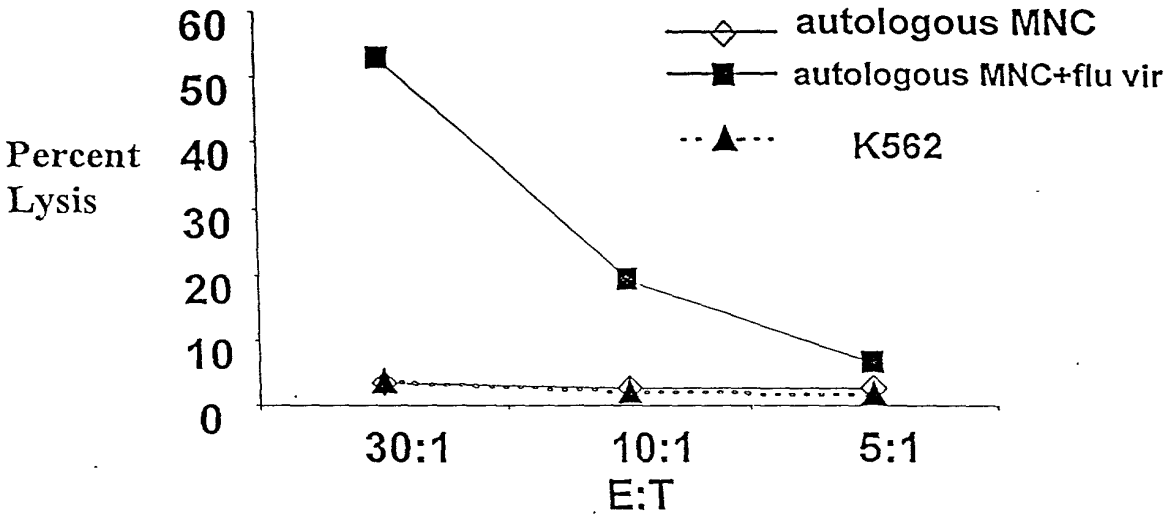


FIGURE 16

CD4+ Primary Cytotoxic T Cell response

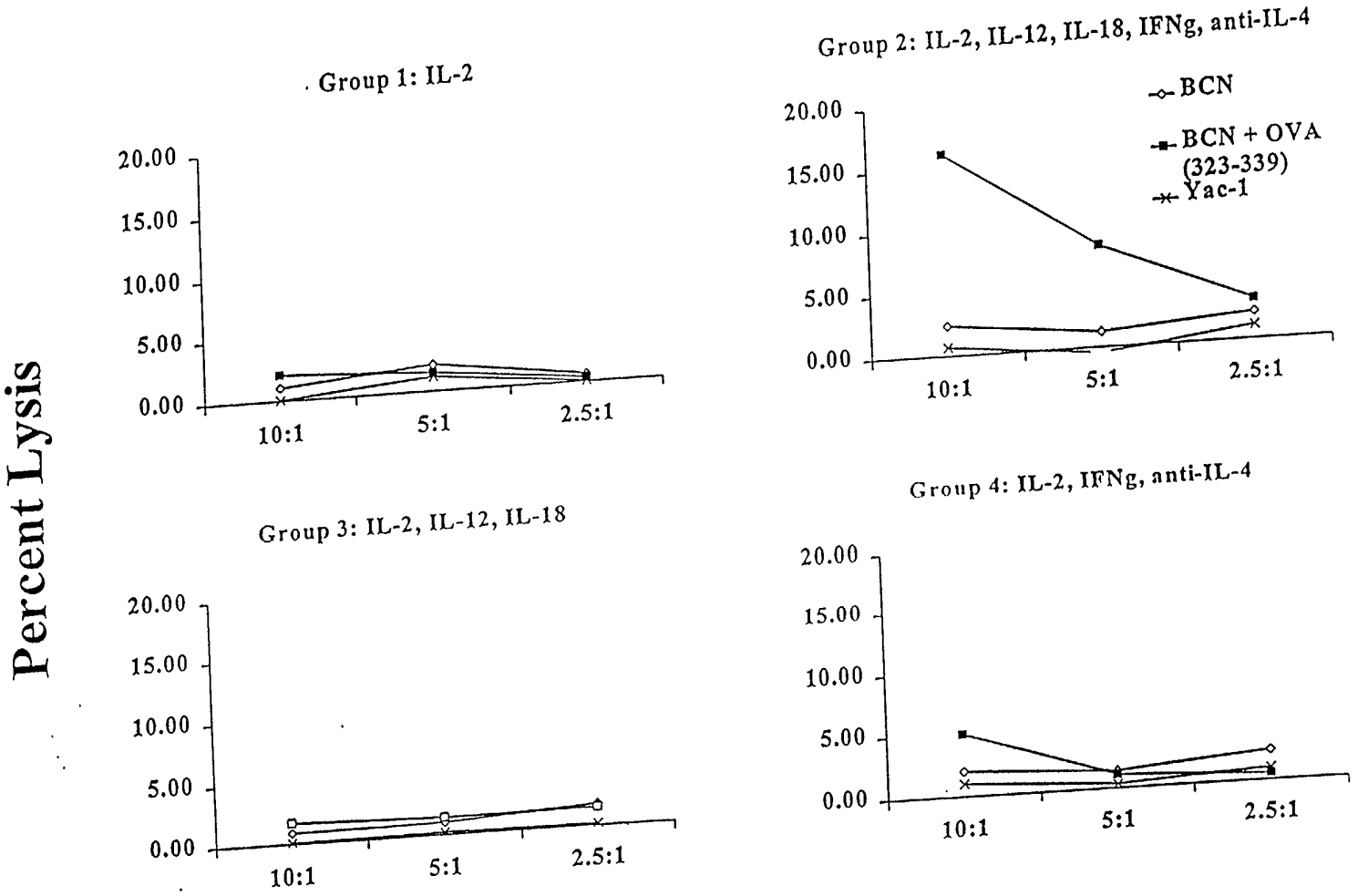


FIGURE 17

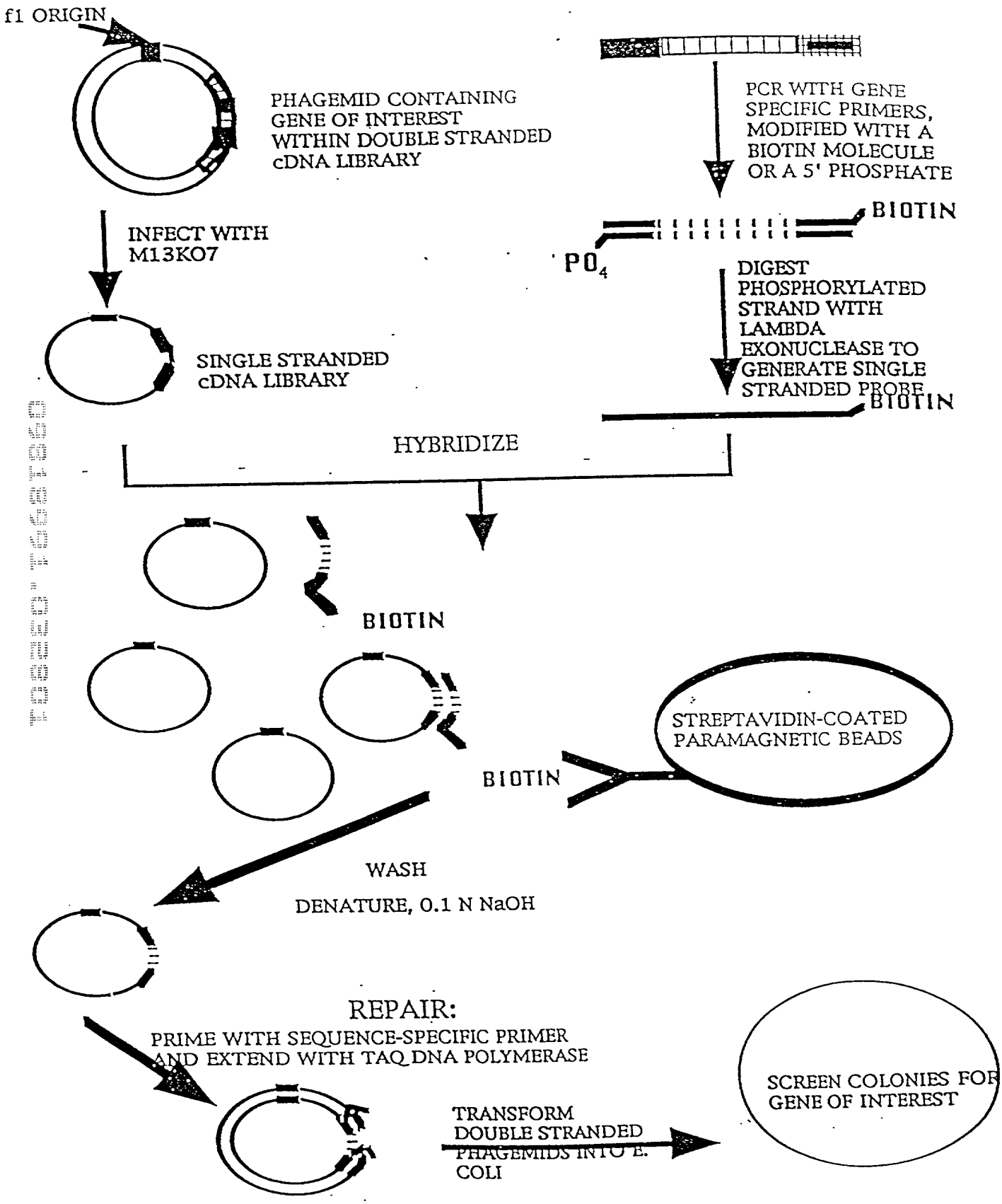

f1 ORIGIN
PHAGEMID CONTAINING GENE OF INTEREST WITHIN DOUBLE STRANDED cDNA LIBRARY
INFECT WITH M13KO7
SINGLE STRANDED cDNA LIBRARY
PCR WITH GENE SPECIFIC PRIMERS, MODIFIED WITH A BIOTIN MOLECULE OR A 5' PHOSPHATE
BIOTIN
PO_4
DIGEST PHOSPHORYLATED STRAND WITH LAMBDA EXONUCLEASE TO GENERATE SINGLE STRANDED PROBE
BIOTIN
HYBRIDIZE
BIOTIN
STREPTAVIDIN-COATED PARAMAGNETIC BEADS
BIOTIN
WASH
DENATURE, 0.1 N NaOH
REPAIR:
PRIME WITH SEQUENCE-SPECIFIC PRIMER AND EXTEND WITH TAQ DNA POLYMERASE
TRANSFORM DOUBLE STRANDED PHAGEMIDS INTO E. COLI
SCREEN COLONIES FOR GENE OF INTEREST

FIGURE 18

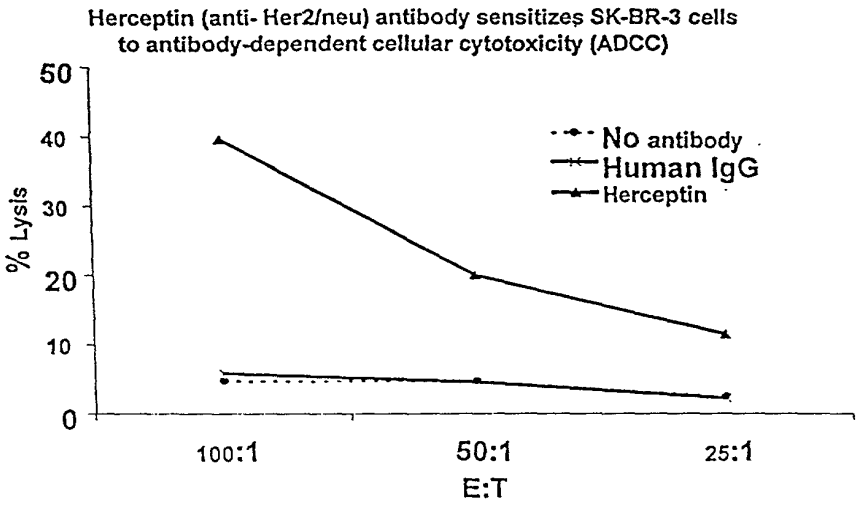

Herceptin (anti- Her2/neu) antibody sensitizes SK-BR-3 cells to antibody-dependent cellular cytotoxicity (ADCC)
50
40
30
20
10
0
% Lysis
No antibody
Human IgG
Herceptin
100:1
50:1
25:1
E:T

FIGURE 19

Tolerance to Alloantigens Induced in presence of Antigens and Anti-CD40 Ligand Antibody

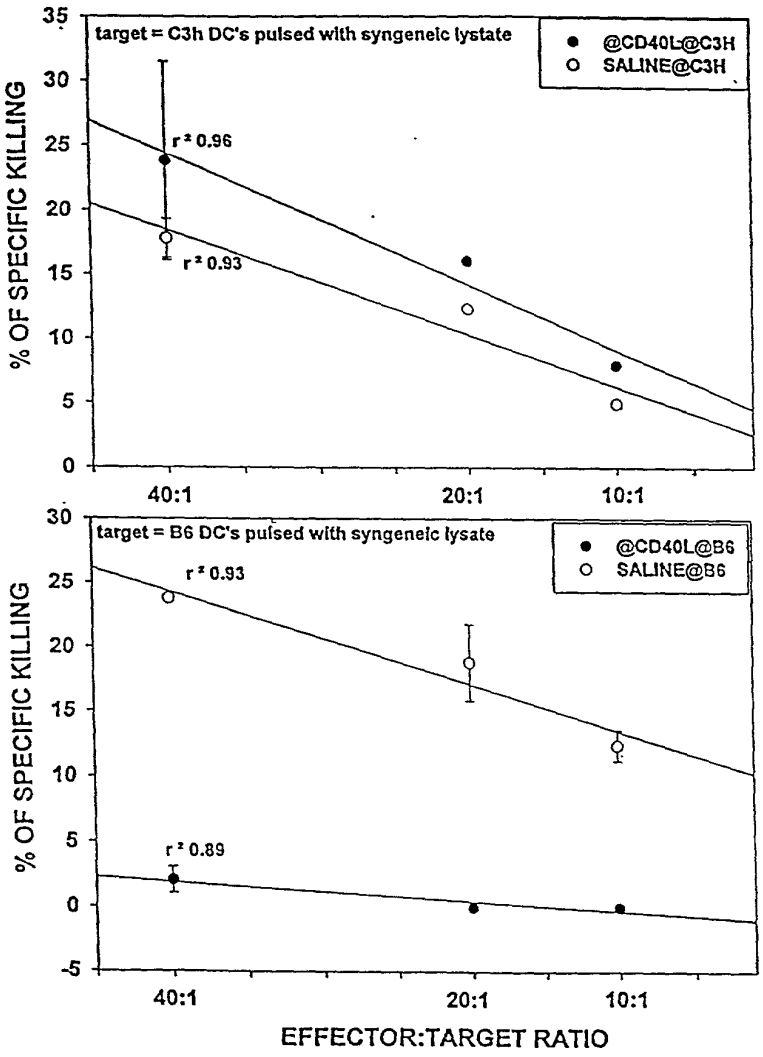


FIGURE 20

Figure 21